GLUTATHIONE METABOLISM IN CORD AND NEWBORN INFANT BLOOD [1, 2]

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A hereditary abnormality of the erythrocytes was described in Negroes, sensitive to primaquine (1). A low level of reduced glutathione (GSH), a GSH instability, as well as a low activity of glucose 6-phosphate-dehydrogenase were demonstrated in those cells (2–5). A similar hereditary abnormality of the erythrocytes has been described in Jews originating from oriental and Mediterranean countries (6–10). It is associated with a susceptibility to hemolysis by various agents such as fava beans, sulpha drugs, para-amino salicylic acid and napthalene. Susceptibility to hemolysis by fava beans was also found in Italians of Sardinian origin and among Greek families residing in the United States (11–15). This hereditary abnormality is transmitted as a sex linked incompletely dominant trait with various degrees of expressivity of the abnormal gene ranging from full to nonpenetrance (9, 15). Zinkham and Childs recently described a nongenetic glutathione instability in the blood of normal newborn infants. This defect was associated with susceptibility to hemolysis by naphthalene as well as by vitamin K (14).

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The present communication summarizes: *a*) studies on the level of GSH and on its stability in the erythrocytes of women during labor and in the umbilical cord blood, and *b*) further investigations on the mechanism of GSH instability in the erythrocytes of newborn infants.

MATERIALS AND METHODS

Blood used was obtained from *a*) women during labor, *b*) umbilical cord, *c*) 30 to 75 hour old infants, and *d*) eight day old infants. Controls were normal blood donors.

All subjects were divided into two ethnographic groups:

1. Ashkenazic Jews (originating from eastern, central and western Europe).
2. Non-Ashkenazic Jews (from oriental and Mediterranean countries).

The latter group was further subdivided according to the country of origin (9). A small number of Arabs was also included in the investigation.

GSH determinations. These determinations were performed on heparinized blood by the method of Grunert and Philips as modified by Beutler and associates (16, 1).

GSH stability test. The method of Beutler was used (2). Five mg. acetylphenylhydrazine per ml. blood or 0.02 mg. menadione sodium bisulfite (vitamin K) in saline was used. Previous investigations reported from this laboratory have demonstrated that when glucose is added to fresh blood the GSH stability test may be performed after 24 hours if blood is kept refrigerated without affecting the results (8, 9).

TABLE I

Mean reduced glutathione (GSH) levels in normal and "sensitive" subjects*

	Normal subjects		Sensitive subjects	
	No. of subjects	Mean GSH*	No. of subjects	Mean GSH*
Cord blood	360	79.7 ± 13.1†	23	82.2 ± 13.4
Women during labor	269	69.6 ± 15.0	28	49.6 ± 10.4
Normal blood donors	219	70.4 ± 13.7	30	50.4 ± 13.5

* GSH in mg. per 100 ml. red blood cells.
† Mean plus or minus standard deviation.

TABLE II

Reduced glutathione (GSH) stability test

Origin of subjects	Sex	Control group*			Women during labor			Cord blood		
		No. of subjects	Sensitives		No. of subjects	Sensitives		No. of subjects	Sensitives	
			No.	*%*		*No.*	*%*		*No.*	*%*
Iraq	M	89	21	23				42	10	24
	F	91	22	24	72	20	28	33	6	18
Yemen	M	62	5	8				21		
	F	50	3	6	41	3	7	24	1	4
North Africa	M	43	1	2				40	1	2.5
	F	40	1	2.5	66	1	1	26		
Egypt	M	19						6	1	16
	F	9			14	1	7	6		
Turkey	M	22	2	9				21		
	F	22	1	5	34	2	6	15	1	6
Bulgaria	M	9						9		
	F	11	1	9	9			3		
Persia	M	9						10	1	10
	F	9	4	44	14	3	21	6	1	16
Others†	M	14	1‡	7				23		
	F	28	3§	10	37			18	1‖	5
Total non-Ashkenazic	M	267	30	11				172	13	8
	F	260	35	13	287	30	10	131	10	7
Arabs	M	40	1	2.5						
	F	13	1	8	3			2		
Ashkenazic	M	203						24		
	F	85			7			28		

* Three hundred four non-Ashkenazic and 288 Ashkenazic subjects summarized in a previous communication (9) are included.

† Including subjects from Greece, Buchara, Syria, Afghanistan and Sudan.

‡ Born in India, grandfather from Iraq.

§ Subjects from Sudan, Aden and one of mixed Greece-Buchara parentage.

‖ Parents from Greece.

The majority of determinations were performed with the addition of glucose (0.1 ml. 4 per cent glucose per ml. of blood). For some of the determinations fresh blood (*i.e.*, assay performed within two hours) without the addition of glucose was used. On the blood of infants a micro method was used. The amount of blood and reagents was reduced by a factor of five.

After deproteinization with 3 per cent metaphosphoric acid, aliquots were centrifuged for 10 minutes at 3,000 rpm in an international centrifuge. The optical density was read in a Klett-Summerson Colorimeter using a green filter and microtubes. The difference between duplicate estimations did not exceed 3 per cent. The results obtained agreed within 5 per cent with those obtained by the macro method.

The term "sensitive" was applied to individuals in whom the erythrocyte GSH level dropped below 30 mg. per 100 ml. red cells as a result of incubation with acetylphenylhydrazine or vitamin K.

Glucose-6-phosphate dehydrogenase activity was estimated. The amount of GSH formed in the following coupled reaction was determined:[3]

$$\text{G-6-P} + \text{TPN}^+ \xrightarrow[\text{dehydrogenase}]{\text{G-6-P}} \text{6-P-G} + \text{TPNH} + \text{H}^+$$

$$\text{GSSG} + \text{TPNH} + \text{H}^+ \xrightarrow[\text{reductase}]{\text{GSH}} 2\text{GSH} + \text{TPN}^+$$

The details of the technique were published elsewhere (10). Six-tenths mg. GSSG was added to a 3 ml. final reaction mixture. The activity of the dehydrogenase was estimated by the amount of GSH formed during a 15 minute incubation at room temperature.

[3] The following abbreviations are used: GSSG, oxidized glutathione; G6P, glucose-6-phosphate; 6PG, 6 phosphogluconate; TPN, triphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide.

TABLE III

Tabulation of "sensitive" subjects according to the reduced glutathione (GSH) level after incubation with acetylphenylhydrazine

Source of blood	GSH level 0–10* Male	0–10* Female	11–20* Male	11–20* Female	21–30* Male	21–30* Female	Total Male	Total Female
Normal blood donors	7	3	4	5	2	9	13	17
Cord blood	5	2	7	3	1	5	13	10
Women during labor		3		10		15		28

* GSH in mg. per 100 ml. red blood cells.

Blood glucose was determined by the method of Hagedorn-Jensen (17).

RESULTS

GSH level in erythrocytes in cord blood, women during labor, and control group

These results are summarized in Table I. No difference in the GSH level was found between the control group (normal blood donors) and normal women during labor, while the mean GSH concentration in cord blood of normal infants was significantly higher ($p < 0.001$).

The mean GSH level in erythrocytes of "sensitive" individuals is known to be lower than the average value for normal subjects. However, in the cord blood no difference in the mean GSH level between "sensitive" and normal infant bloods was found. All subjects with GSH instability had normal GSH concentration in the blood obtained from the umbilical cord.

GSH stability test on bloods obtained from umbilical cord, women during labor, and control group

The results are summarized in Table II (592 subjects belonging to the control group and presented in an earlier publication are included in the table). No cases with GSH instability were found among the Ashkenazic subjects, while the mean frequency of GSH instability in the non-Ashkenazic groups was about 9 per cent. No significant differences were found in the frequency and ethnographic distribution of the erythrocyte GSH instability between cord blood and the other two investigated groups. In all three groups subjects originating from Iraq showed the highest frequency of the abnormality (above 20 per cent).

The observation that the GSH levels in "sensitive" males were usually lower than in "sensitive" females when their erythrocytes were incubated with acetylphenylhydrazine was found to be valid for the cord blood also (see Table III).

GSH stability in young infants

All tests presented in this section were performed by the micro method using fresh blood without the addition of glucose. The results are presented in Table IV.

All infants 30 to 75 hours old so far studied demonstrated a GSH instability, although cord blood from the same infants had stable GSH. The GSH instability was no longer demonstrable

TABLE IV

Reduced glutathione (GSH) stability in infants according to age

Source of blood	GSH* in fresh blood	GSH after incubation with APH†	GSH after incubation with Vitamin K
Cord blood			
With stable GSH	a) 90‡	82	80
	b) 89	56	60
	c) 83	60	56
	d) 67	56	58
With unstable GSH	84	11	15
	93	24	22
Infants, 30 to 75 hours old	a) 85‡	5	
	b) 90	6	
	c) 90	16	15
	d) 75	18	13
8 days old	84	51	60
	65	44	40
	102	65	

* GSH in mg. per 100 ml. red blood cells.
† Acetylphenylhydrazine.
‡ a, b, c, d, the same subjects examined at birth (cord blood) and at the age of 30 to 75 hours.

TABLE V

Influence of glucose and inosine on reduced glutathione (GSH) stability of blood obtained from umbilical cord, infants and adults

Source of blood	The fresh blood		GSH* after incubation with APH‡		
	GSH*	Glucose†	No glucose added	Glucose	Inosine
				4 mg./ml. blood	*5 mg./ml. blood*
GSH instability (hereditary)					
Cord blood	75	100	8	8	
	85	88	15	17	
	91	90	5	9	
Adult blood	38	117	8	8	7
	48	94	15	13	17
Infants (normal)					
30–75 hours old	90	79	16	80	
	75	65	15	60	
	80	69	20	89	92
	70		12	59	55
	102		19	98	80
8 days old	84	74	51	50	52
	65	79	44	70	54
	102		65	100	87

* GSH in mg. per 100 ml. red blood cells.
† Glucose in mg. per cent.
‡ Acetylphenylhydrazine.

in eight day old infants. The GSH instability was present in infants of Ashkenazic and non-Ashkenazic descent.

The effect of glucose and inosine on this GSH instability is summarized in Table V. Glucose as well as inosine have normalized the GSH instability in infants' blood, but had no effect on the hereditary type of GSH instability.

TABLE VI

Results of glucose-6-phosphate dehydrogenase assay *

Subjects	GSH (mg./3 ml. mixture) formed by reduction of GSSG
	mg.
Adults with stable GSH	0.25
	0.33
	0.38
Adults with GSH instability	0.04
	0.10
Infant 36 hours old	0.54
Infant 42 hours old	0.48
Cord blood with stable GSH	0.43
	0.40
Cord blood with GSH instability	0.02

* Incubation mixture contained 0.6 mg. GSSG.

This *in vitro* effect of glucose on the erythrocyte GSH instability in infants could be related to a lower glucose level in the newborn infants' blood. The glucose levels in the blood obtained from the umbilical cord, newborn and adult, are presented in Table V.

Although the glucose level in fresh blood obtained from 30 to 75 hour old infants was lower than in cord blood, the eight day old infants had blood glucose levels similar to those found in 30 to 75 hour old infants. Another explanation for the effect of glucose could be an increased utilization of glucose by the erythrocytes of the newborn. This is currently being investigated.

Glucose-6-phosphate dehydrogenase activity

In contrast to the hereditary type of GSH instability the enzyme activity in infant blood was not found to be decreased (see Table VI). These results confirm the data obtained by Gross and Hurwitz (18).

DISCUSSION

The results presented in this communication indicate that the hereditary abnormality of the erythrocytes which occurs in certain Jewish communities (and probably in other "races") manifests

itself already during fetal life. These cells demonstrate a GSH instability *in vitro,* a decreased G-6-P dehydrogenase activity and some reduction in GSH concentration. Recently an increase in glutathione reductase was demonstrated (19). Preliminary investigations in this laboratory indicate a decreased incorporation of glycine 1-C-14 into glutathione of "sensitive" erythrocytes *in vitro* (20).[4]

The mechanism of the *in vitro* erythrocyte GSH instability in 30 to 75 hour old infants is completely different from the inherited GSH instability. This erythrocyte GSH instability is not inherited nor is it racially determined. It appears only after birth and is very transient in nature. It is not accompanied by a decrease in G-6-P dehydrogenase activity. Furthermore, it may be prevented by raising the blood glucose or by the addition of inosine to the incubation medium. In this respect it is similar to the GSH instability appearing in normal blood in which the glucose content has been artificially lowered either by incubation for 24 hours at room temperature or by suspending washed erythrocytes in saline or buffer solution devoid of glucose (2, 3, 8). Glycine 1-C-14 incorporation into the erythrocyte glutathione of those newborns is normal, while in the hereditary abnormal erythrocyte the glycine incorporation is low (20).[4] The glucose level in the blood of young infants did not differ much from that found in cord blood or eight day old infants' blood. In spite of these *in vitro* results, the effect of low blood glucose concentration on GSH instability in newborns cannot be completely discarded at present. The physiological hypoglycemia is generally more pronounced and of longer duration in prematures than in normal termed infants (21, 22). Most hemolytic reactions due to vitamin K administration have been described in premature infants (23, 24). Hemolysis following naphthalene ingestion or unidentified agents in such infants is also known (25, 26). It is even possible that such drugs administered to nursing mothers could be transmitted through the milk to the infants and cause hemolysis in the infant in analogy to cases of favism caused by milk of goats fed fava beans as mentioned by Luisada (27).

The mechanism by which glucose or inosine exhibit a protective effect upon the erythrocyte GSH instability is not clear.

Glucose probably enters the cell via an active process which is catalyzed by hexokinase whose coenzyme is adenosine triphosphate (ATP). The glucose is further metabolized via the glycolytic pathway and probably to some extent via the hexose monophosphate shunt (28, 29). Beck has shown that the extent to which white blood cells metabolize glucose via the shunt mechanism is directly determined by the glucose-6-phosphate level in the cells and indirectly by the hexokinase activity (30). It is possible that a similar mechanism operates in the erythrocytes also.

The hexokinase activity and the ATP level in the red cell of the newborn infants is under investigation.

The normal GSH stability in cord blood in comparison with its instability during the first days of the infant's life could be the result of an increased permeability of glucose into the fetal red cells *in utero.* Those possibilities are being further investigated.

SUMMARY

The reduced glutathione level and its stability has been studied in women during labor and in cord blood. The mean glutathione level in cord blood was found to be higher than in adult bloods. The hereditary abnormality of the erythrocyte leading to susceptibility to hemolysis was already detectable in cord blood. In the Jewish population, this abnormality was present in non-Ashkenazics only, the highest frequency being encountered among those of Iraqi origin. The reduced glutathione level in cord blood of "sensitive" subjects was within normal limits.

In normal newborn infants a transient, nonhereditary glutathione instability appears during the first hours of life. It is not associated with defective glucose-6-phosphate dehydrogenase activity. This erythrocyte reduced glutathione instability may be prevented by the addition of glucose or of inosine to the blood *in vitro.* The pos-

[4] Additional investigations of glycine 1-C-14 incorporation rate into erythrocyte glutathione (GSH) suggested that the low specific activity of GSH isolated from the "sensitive" erythrocytes could be caused not by low glycine incorporation rate, but by the rapid disappearance of GSH from these cells due to the presence of cystein in the incubation mixture. The experimental details will be published.

sible mechanism of the nonhereditary reduced glutathione instability is discussed.

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